

BOCADEVA 0714

Gulf of Cadiz Anchovy Egg Survey and 2014 SSB preliminary estimates.

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Introduction

The Daily Egg Production Method (DEPM) to estimate the anchovy spawning stock biomass (SSB) in the Gulf of Cádiz ((ICES, Subdivision IXa South) is conducted every three years by IEO (Spain). The first survey of this series was carried out in 2005 (Jiménez *et al.*, 2005). The DEPM survey *BOCADEVA 0714* (the fourth Anchovy DEPM survey in the series) is one of the research activities developed in 2014 under the project ICTIOEVA12 (*Métodos de Producción de Huevos, Estimación de la biomasa de especies pelágicas de interés comercial: sardina, anchoa, caballa y jurel*).

The survey has been carried out on board R/V *Ramón Margalef* (IEO) from 24st to 31 July 2014. The survey dates are determinate by the reproductive cycle of the species in the study area, and they should coincide with the maximum peak spawning.

The surveyed area extended from Strait of Gibraltar to Cape San Vicente (Spanish and Portuguese waters in the Gulf of Cadiz). Plankton samples, along a grid of parallel transects perpendicular to the coast, are obtained for the spawning area delimitation and density estimation of the daily egg production. The fishing hauls for estimation of adult parameters (sex ratio, female mean weight, batch fecundity and spawning fraction) are undertaken in the ECOCADIZ 201407 survey, carried out during the same period.

The survey objectives also included to characterize oceanographic and meteorological conditions in the study area during the survey

This working document provides a brief description of the survey, laboratory analysis and estimation procedures used to obtain the Gulf of Cadiz anchovy SSB by DEPM for 2014 in the South-Atlantic Iberian Stock. Results are preliminary, because the estimation of the spawning fraction is not available yet.

Methodology

Table I presents a summarised description of the methodology used to obtain eggs and adults samples. Sampling grid was established in 21 transects perpendicular to the coast, 8 nm between transects and 3 nm between stations (*Study Group on Spawning Biomass of Sardine and Anchovy*, ICES 2003).

Table I. *BOCADEVA 0714*. Gulf of Cadiz Anchovy DEPM survey. General sampling.

Eggs Parameters	Anchovy DEPM survey <i>BOCADEVA0711</i>
Survey area	(36°13'–36°50'N –6°07'–8°55'W)
R/V	<i>Ramón Margalef</i>
Date	24-31 July
Transects (Sampling grid)	21 (8x3)
Pairovet stations (150 µm)	151
Sampling maximum depth (m)	100
Hydrographic sensor	CTD SBE25PLUS and mini CTD Valeport
Flowmeter	Yes
CUFES stations	153
CUFES (335µm)	3 n miles (sample unit)
Environmental data	Temperature and Salinity
Adults Parameters	
Survey area	(36°11'–36°47'N –6°12'–8°54'W)
R/V	<i>Miguel Oliver</i>
Date	24/07-06/08
Gears	Pelagic trawl
Trawls	25 (1 null; 23 positive for anchovy)
Trawls time	From 07:15 to 19:46 hrs GMT
Biological sampling:	On fresh material, on board of the R/V
Sample size	At least 60 individuals randomly picked; up to 120 (adding batches of 10 randomly picked anchovies) if a minimum of 30 mature females were not found for spawning fraction estimation. A minimum of 150 hydrated females for batch fecundity estimation.
Fixation	4% Phosphate buffered Formaldehyde
Preservation	4% Phosphate buffered Formaldehyde

Egg sampling and processing

The strategy of egg sampling was identical to that used in previous *BOCADEVA* surveys. An adaptive sampling was carried out in the E-W direction using a PairoVET net in fixed stations as main sampler and a continuous recording with CUFES (Continuous Underwater Fish Egg Sampler) as secondary sampler.

▪ *Vertical sampling (PairoVET)*

The sampling grid was established on the continental shelf following a systematic sampling scheme, with the transects being perpendicular to the coast and equally spaced 8 nm. Egg samples were always taken every 3 nm in the inner shelf, up to 100 m depth (ICES, 2003). The inshore limit of transects was determined by bottom depth (as close to the shore as possible), while the offshore extension was decided adaptively depending on the results of the most recent CUFES sample.

Vertical hauls of plankton were carried out with a PairoVET sampler equipped with nets of 150 μm of mesh size. Hauls were carried out up to a maximum depth of 100 m or of 5 m above the bottom in shallower depths, with a speed of about 1 m/s. Sampling depth and temperature of the water column were recorded using a mini CTD Valeport fitted to the net. Flowmeters were used to calculate the volume of filtered water during each haul. Egg samples were analysed onboard. A preliminary identification and counting of anchovy eggs and larvae, as well as other commercial species were carried out. Samples were sorted, counted and preserved in a 4 % buffered formaldehyde solution. In the laboratory, anchovy eggs were classified in 11 developmental stages, according to the key proposed by Moser and Ahlstrom (1985).

▪ *Continuous sampling (CUFES)*

During the CUFES sampling (Checkley *et al.*, 2000) the volume of filtered water (600 l/min, approximately) was also integrated each 3 nm (at a fixed depth of 5 m). The CUFES collector was arranged with a 335 μm net. Anchovy eggs were classified in three stages: No-Embryo (I-III), Early Embryo (IV-VI) and Late Embryo (VII-XI).

Adult sampling and processing

Adult anchovy samples for DEPM purposes were obtained during the ECOCADIZ 201407 survey from pelagic trawl hauls (Ramos *et al.*, 2014).

Except for searching anchovy females with hydrated gonads, fishing stations were mostly conducted during daylight hours and carried out over isobath, once echotracess supposedly belonging to anchovy were detected by echo-sounder.

For the estimation of spawning fraction (S), a minimum of 30 mature, non-hydrated females per sample is sought, so a minimum of 60 random anchovies are sampled, adding batches of 10 random individuals to the sampling until the goal is achieved or a maximum of 120 anchovies are sampled. Sex-ratio (R), along with other parameters used in the DEPM is also obtained from this random sampling.

When hydrated females (HF) appeared, an additional sampling was done in order to obtain a minimum of 150 HF for the whole area prospected. These females were sampled as described above. Gonads from both hydrated and non-hydrated females were preserved in 4% buffered formaldehyde.

Mean female weight (W) was estimated after correction for the increase in weight due to the hydration in hydrated females. Sex ratio (R) was estimated as the weight ratio of females in the mature population.

The individual batch fecundity (F_{obs}) was estimated by the hydrated oocyte method (Hunter *et al.*, 1985). The spawning fraction (S) is currently being determined by histological analysis of the post-ovulatory follicles, POFs (Hunter and Macewicz, 1985). Post-ovulatory follicles (POF's) were assigned to stages-ages according to the Motos's classification (1996) (Day-0 POFs (0-6 h); Day-1 POFs (7-30 h); Day-2 POFs (31-54 h); Day-2+ POFs (older than 54 h), although considering as the peak spawning time the species-specific for the study area.

Data analysis and estimation

▪ *Egg Production (z , P_0 and P_{tot}) estimation and area calculation*

All calculations for area delimitation, egg ageing and model fitting for egg production (P_0) estimation were carried out using the R packages *geofun*, *spatstat*, *eggsplore* and *shachar* available at *ichthyoanalysis* (<http://sourceforge.net/projects/ichthyoanalysis>).

The surveyed area (A) was calculated as the sum of the area represented by each station. The spawning area ($A+$) was delimited with the outer zero Anchovy egg stations, and was calculated as the sum of the area represented by those stations. The model of egg development with temperature was derived from the incubation experiment carried out in Cádiz in July 2007 (Bernal *et al.*, 2012). A multinomial model was applied (Ibaibarriaga *et al.*, 2007, Bernal *et al.*, 2008) considering only the interaction Age*Temp (other interactions were not significant).

$$N_{i,t} \sim \text{Mult} (N, p_{i,t})$$

$$p_{i,t} = f(\text{Age}, \text{Temp})$$

Egg ageing was performed by a multinomial Bayesian approach described by Bernal *et al.* (2008) and using *in situ* SST; a normal probability distribution was used with peak spawning assumed to be at 22:00h with 2h standard deviation. This method uses the multinomial development model and the assumption of probabilistic synchronicity (assuming a normal distribution).

$$p(\text{age} \mid \text{stage}, \text{temp}, \text{time}) \propto p(\text{stage} \mid \text{age}, \text{temp}) p(\text{age} \mid \text{time})$$

ageing development model synchronicity

Daily egg production (P_0) and mortality (z) rates were estimated by fitting an exponential mortality model to the egg abundance by cohorts and corresponding mean age. The model was fitted using a generalized linear model (GLM) with negative binomial distribution. The ageing process and the GLM fitting were iterative until the value of z converged. [depm.control (spawn.mu=22; how.complete=0.95; spawn.sig=2), initial $z = 0.01$].

$$P_{age} = P_0 e^{-z age}$$

$$\log\left(\frac{N_{age}}{area}\right) = \log(P_0) - z age \rightarrow \log(N_{age}) = \log(area) + \log(P_0) - z age$$

Finally, the total egg production was calculated as: $P_{tot} = P_0 A +$

▪ *Adult parameters*

The adult parameters estimated for each fishing haul considered only the mature fraction of the population (determined by the fish macroscopic maturity data).

Before the estimation of the mean female weight per haul (W), the individual total weight of the hydrated females was corrected by a linear regression between the total weight of non-hydrated females and their corresponding gonad-free weight (Wnov). The sex ratio (R) in weight per haul was obtained as the quotient between the total weight of females and the total weight of males and females. The expected individual batch fecundity for all mature females (hydrated and non-hydrated) was estimated by modelling the individual batch fecundity observed (Fobs) in the sampled hydrated females and their gonad-free weight (Wnov) by a GLM. The fraction of females spawning per day (S) is determined, for each haul, as the average number of females with Day-1 or Day-2 POF, divided by the total number of mature females (the number of females with Day-0 POF is corrected by the average number of females with Day-1 or Day-2 POF, and the hydrated females are not included).

The mean and variance of the adult parameters for all the samples collected was then obtained using the methodology from Picquelle and Stauffer (1985; *i.e.*, weighted means and variances). All estimations and statistical analysis were performed using the R software v.2.8.1.

▪ *Spawning Stock Biomass*

The spawning Stock Biomass was computed according to:

$$SSB = \frac{P_{total} * W}{F * S * R}$$

However, the SSB estimates for 2014 should still be considered with caution because the spawning fraction parameter (S) has not been estimated yet, using instead as two alternatives: 1) the 2011 value estimate for this parameter; 2) the mean of the S 2008-2011 values.

Results

The surveyed area (14595 km²) extends from Cabo de Trafalgar (Spain) to Cabo de San Vicente (Portugal), from (36°13'–36°50'N –6°07'–8°55'W). This area includes the continental shelf of the Gulf of Cadiz. The survey was carried out from East to West, starting in the radial 1- station 1, located close the Strait of Gibraltar (**Fig. 1**).

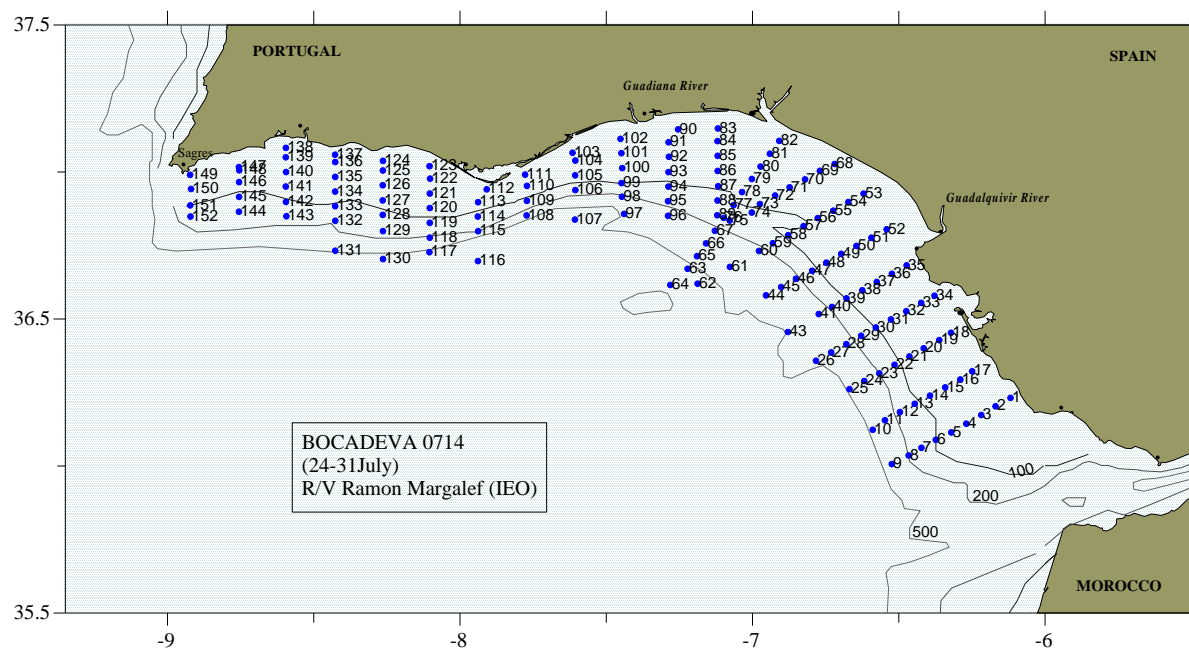


Figure 1. BOCADEVA 0714 survey. PaïroVET stations locations.

Distribution and abundance of anchovy eggs

The ichthyoplankton sampling almost covered the whole 24 hours' day-time period. A total of 151 PaïroVET stations were carried out. In 70 stations (46.43%) there was presence of anchovy eggs (positive stations). A total of 3097 anchovy eggs were caught, and a maximum density (in number/m²) of 2024.4 was obtained (**Table II**). Only 16 Sardine eggs were caught.

Table II. BOCADEVA 0714. Number and density of anchovy eggs sampled by the PaïroVET net during the survey.

By PaïroVET	Anchovy eggs
N stations	151
N positive stations	70
N total eggs	3097
N medium eggs	20.4
N máximo eggs	195
Total density (eggs/m ²)	33019
Mean density (eggs/m ²)	218.7
Maximum density (eggs/m ²)	2024.4

Anchovy eggs were caught mainly in the coastal area located between the radial 3 and 12 and the radial 17, in Portuguese waters (**Fig. 2**). High abundances were also found in stations located close to Huelva. In these stations (all of them with a density > 1000 eggs/m² and located inside isobaths of the 130 m) the temperature (SST) ranged between 17.9 and 23.6 °C (mean 21.6 °C). In the total area, the SST ranged between 15.1 and 23.9 °C (mean 20.6 °C), very similar to 2011 (**Fig. 2**).

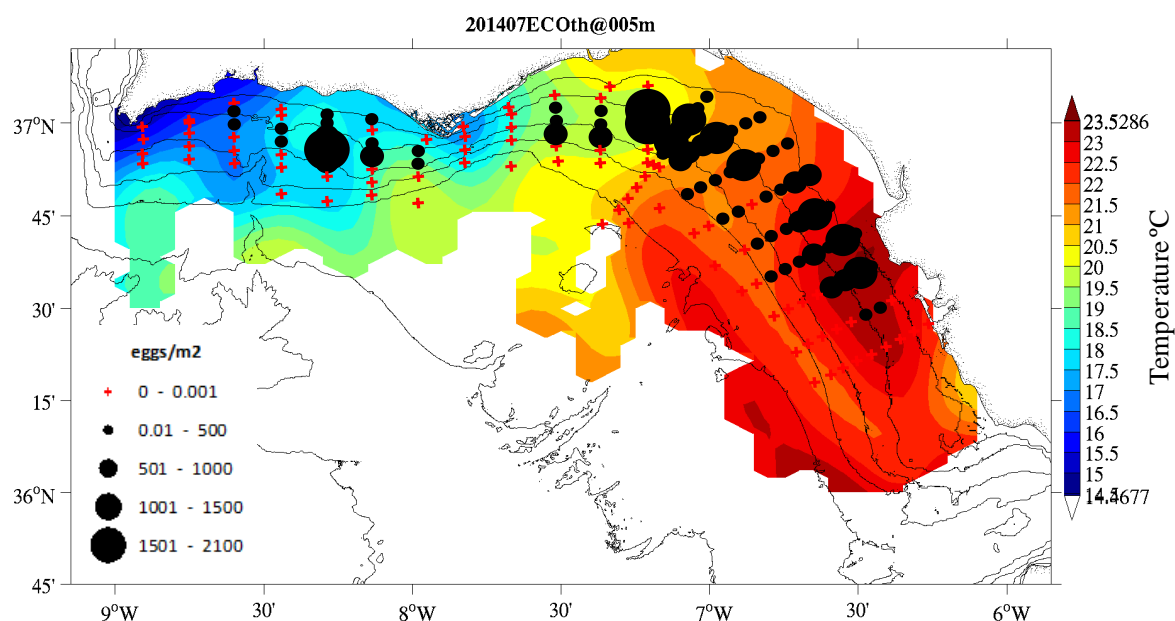


Figure 2. Gulf of Cadiz anchovy DEPM 2014 survey. Abundance distribution of anchovy eggs sampled with PairoVET and SST.

98.5% of the anchovy eggs have been classified into 11 stages according to the degree of embryonic development. Eggs in stage I have not been found. The most abundant development stages were II (32.4%), and IX and VI (14.8 and 11.7%, respectively). XI stage eggs, right before the hatching, represented 0.6% (**Fig. 3**).

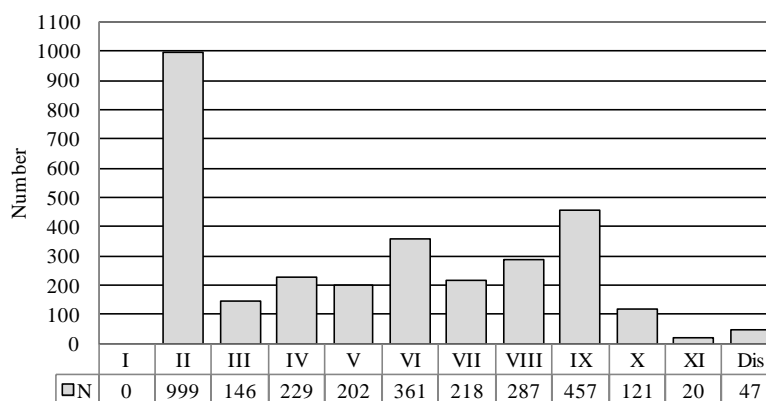


Figure 3. Gulf of Cadiz anchovy DEPM 2014 survey. Number of anchovy eggs classified into the different developmental stages (PairoVET).

Eggs in Stage II were caught between 22:56 and 13:44 hrs GMT, approximately, with a maximum peak of abundance about 05:21 hrs GMT (**Fig. 4**), coincident with the peak spawning for this species in the GoC, which is fixed at 22:00 hrs GMT (Jimenez *et al.*, 2009).

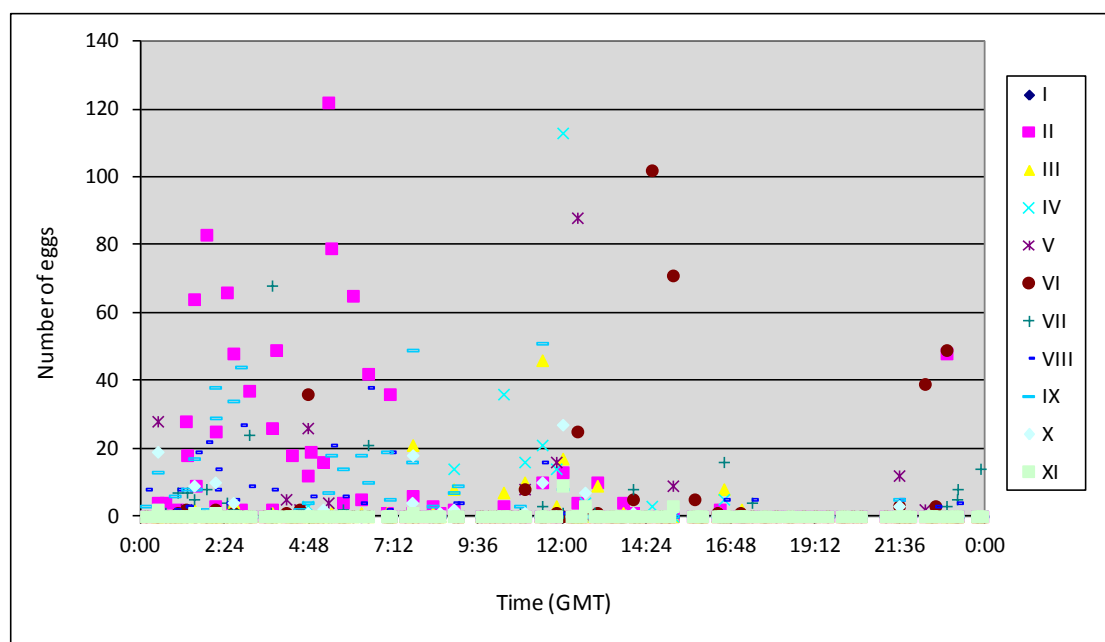


Figure 4. Gulf of Cádiz Anchovy DEPM 2014 survey. Number of eggs caught by development stage by the sampling time (PairoVET).

Adults. Results of the pelagic hauls

See Ramos *et al.*, 2014.

Eggs parameters

The cumulative plot of the total dens and temperature by range of temperature is show in **Fig. 5**. The mean temperature into the 0-10 m stratum has been used for the estimates. Daily egg production (P_0) and mortality (z) rates were estimated by fitting an exponential mortality model to the egg abundance by cohorts and corresponding mean age (**Fig. 6**). The model was fitted using a generalized linear model (GLM) with negative binomial distribution (**Table V, Fig. 7**). The ageing process and the GLM fitting were iterative until the value of z converged. [depm.control (spawn.mu=22; how.complete=0.95; spawn.sig=2), initial $z = 0.01$].

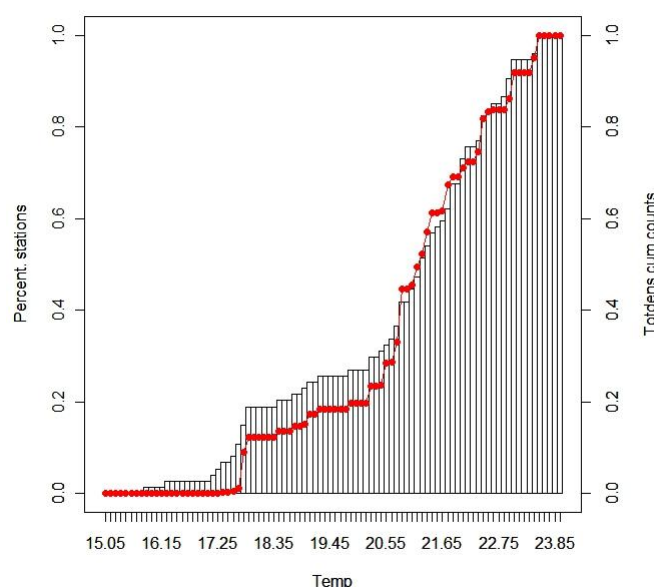


Figure 5. Cumulative plot of total dens and temperature by range of temperature (inter=0.1)

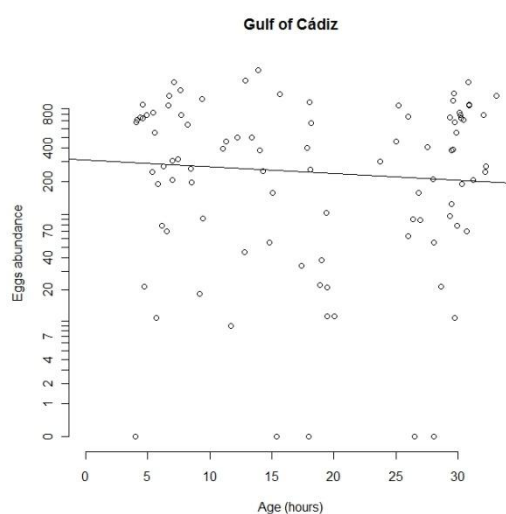


Figure 6. Gulf of Cádiz Anchovy DEPM 2014 survey. Exponential mortality model.

Table V. Gulf of Cádiz Anchovy DEPM 2014 survey. Egg production and mortality. Selected Generalized lineal model (GLM).

```

glm.nb(formula = cohort ~ offset(log(Efarea)) + age, data = aged.data,
       weights = Rel.area, init.theta = 0.446838357531435, link = log)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-1.9229  -1.2004  -0.4613   0.3059   1.4731

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  5.74784    0.34859  16.489  <2e-16 ***
age         -0.01389    0.01657  -0.838    0.402
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(0.4468) family taken to be 1)

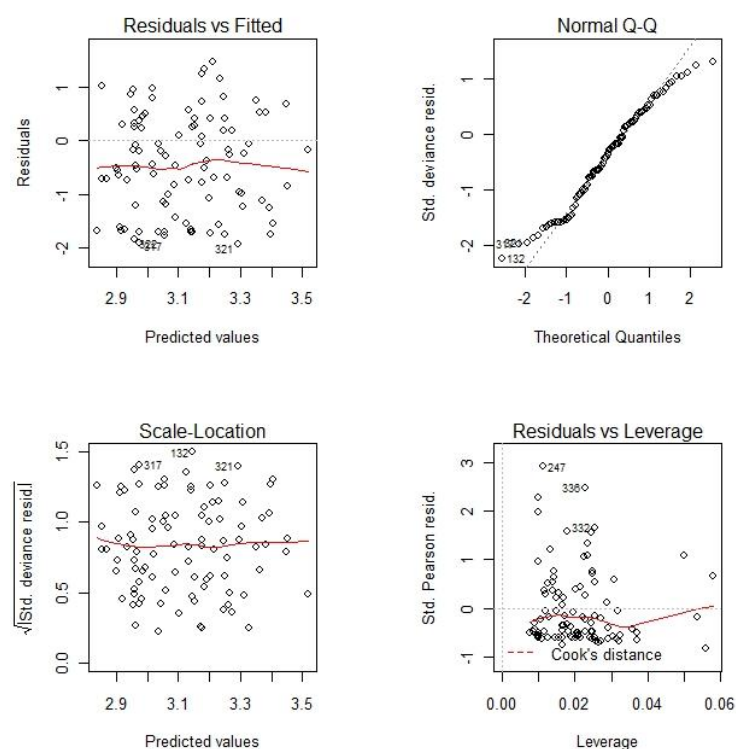
Null deviance: 98.34  on 94  degrees of freedom
Residual deviance: 97.66  on 93  degrees of freedom
AIC: 662.47

Number of Fisher Scoring iterations: 1

            Theta: 0.4468
            Std. Err.: 0.0690

2 x log-likelihood: -656.4690

```

**Figure 7.** Gulf of Cádiz Anchovy DEPM 2014 survey. Residual inspection plots for the Generalized Linear Model fitted to Anchovy egg production data.

Adult parameters by haul

The total weight of hydrated females was corrected for the increase of weight due to the hydration process by a linear regression model between individual data of gonad-free-weight (Wnov) and its corresponding total weight (Wt) from non-hydrated females (**Table VI, Fig. 8**).

Table VI. Gulf of Cádiz Anchovy DEPM 2014 survey. Results of the linear regression model for the relationship between non-hydrated females total weight (Wt) and ovary-free weight (Wnov).

```
lm(formula = Wt ~ Wnov, data = adults.dat[which.weight, ])

Residuals:
    Min       1Q   Median       3Q      Max
-1.22006 -0.17345 -0.01925  0.13338  1.26607

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept) -0.136729   0.032988  -4.145 3.84e-05 ***
Wnov         1.068078   0.001786 598.171 < 2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.3013 on 671 degrees of freedom
Multiple R-squared:  0.9981,    Adjusted R-squared:  0.9981
F-statistic: 3.578e+05 on 1 and 671 DF,  p-value: < 2.2e-16
```

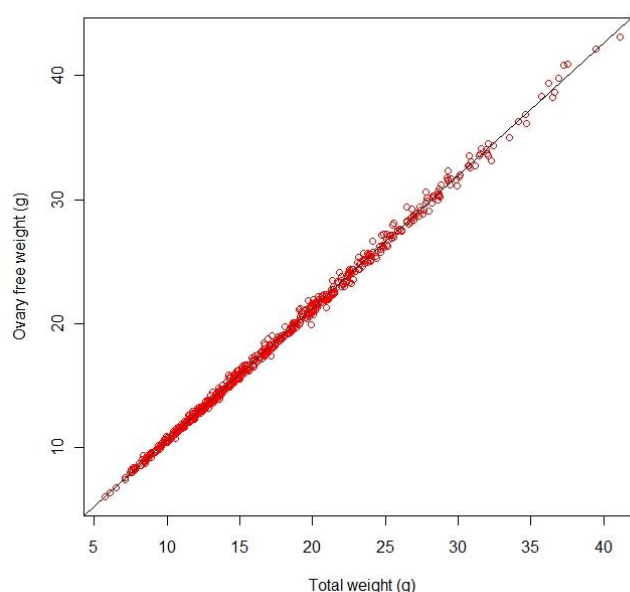


Figure 8. Gulf of Cadiz anchovy DEPM 2014 survey. Plot of the linear regression model for the relationship between non-hydrated females total weight (Wt) and ovary-free weight (Wnov).

The expected female weight (Wexp) for all mature females was also estimated using this linear regression model.

The expected batch fecundity for all mature females (Fexp) was estimated by modelling the observed individual batch fecundity (Fobs) in hydrated females in function of their gonad-free-weights (Wnov)

by a GLM model (**Fig. 9**). Results of this model and the residual inspection plots are shown in **Table VII** and **Fig. 10**.

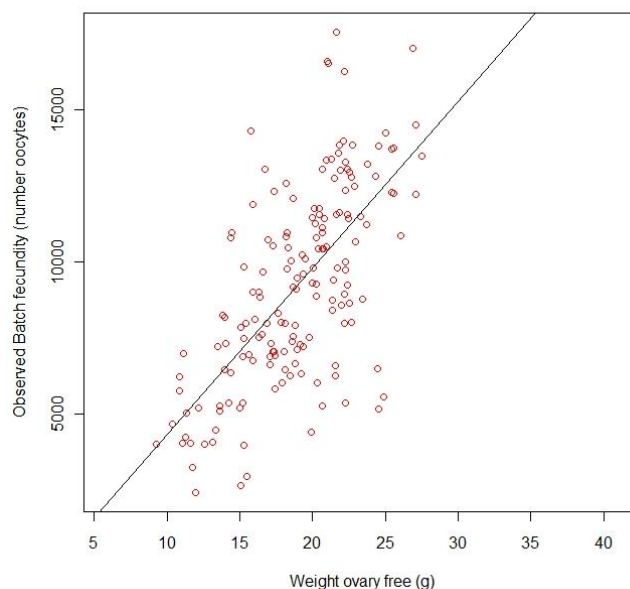


Figure 9. Gulf of Cadiz anchovy DEPM 2014 survey. Generalized linear model for the relationship between observed individual batch fecundity (Fobs) and ovary-free weight (Wnov).

Table VII. Gulf of Cadiz anchovy DEPM 2014 survey. Batch fecundity. Selected Generalized linear model (GLM).

```
glm.nb(formula = Fobs ~ Wnov, data = adults.dat, na.action = "na.omit",
  link = identity, init.theta = 12.8447839708990)

Deviance Residuals:
    Min       1Q   Median       3Q      Max
-3.032258  -0.685285   0.005756   0.541384   2.599268

Coefficients:
            Estimate Std. Error z value Pr(>|z|)
(Intercept) -1176.20     737.26  -1.595    0.111
Wnov         549.08      42.86  12.810 <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

(Dispersion parameter for Negative Binomial(12.8448) family taken to be 1)

Null deviance: 296.48  on 166  degrees of freedom
Residual deviance: 169.19  on 165  degrees of freedom
(1322 observations deleted due to missingness)
AIC: 3084.4

Number of Fisher Scoring iterations: 1

            Theta: 12.84
        Std. Err.: 1.39

2 x log-likelihood: -3078.432
```

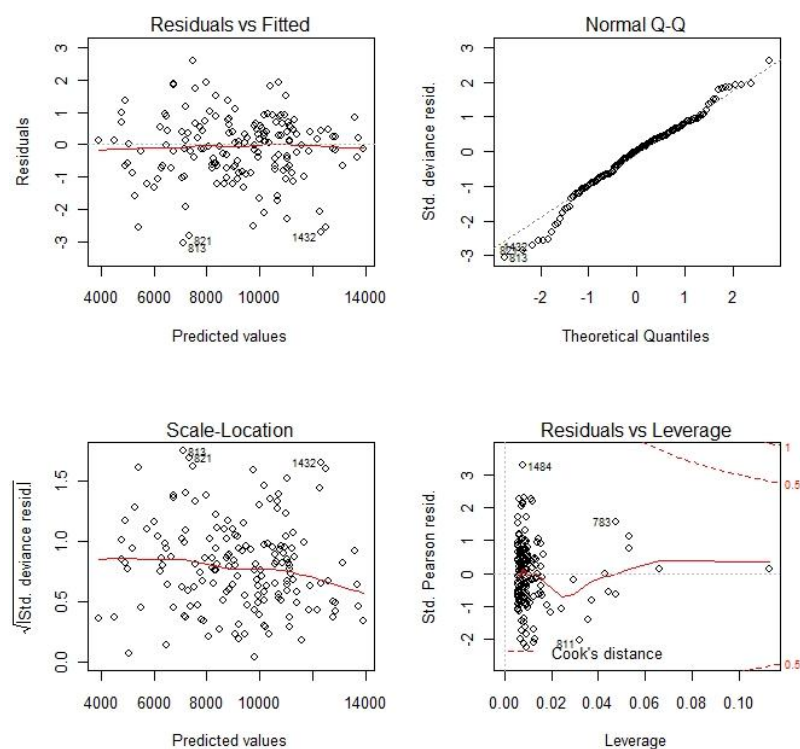


Figure 10. Gulf of Cadiz anchovy DEPM 2014 survey. Residual inspection plots for the Generalized Linear Model fitted to the anchovy batch fecundity data.

Preliminary SSB 2014 estimates

The total spawning area ($A+$) was 6214 Km². The spawning fraction (S) has not been estimated yet. In order to obtain a preliminary estimate of the SSB for 2014 two alternatives has been tested: 1) SSB1: S_1 = derived from the mean 2008 and 2011 S values; 2) SSB2: S_2 = derived from the 2011 S value.

The values of the mean estimates and their associated variances for the egg and adult parameters, and the preliminary SSB are summarized in the **Table VIII**, and the historical series is shown in **Table IX**.

Table VIII. Gulf of Cadiz anchovy DEPM 2014 survey. Summary of the results for eggs, adults and a preliminary SSB estimates (CVs in brackets).

Parameters	Gulf of Cádiz 2014
Eggs	
P_0 (eggs/m ² /day)	313.5 (0.34)
Z (day ⁻¹)	-0.33 (1.19)
P_{tot} (eggs/day) ($\times 10^{12}$)	1.95 (0.34)
Positive area (Km ²)	6214
Adults	
Female Weight (g)	18.22 (0.08)
Batch Fecundity	7502 (0.08)
Sex Ratio	0.54 (0.008)
Spawning Fraction 1	0.247
Spawning Fraction 2	0.276 (0.04)
SSB 2014	
Spawning Stock Biomass 1 (tons) (CV)	35275 (0.30)
Spawning Stock Biomass 2 (tons)	31569 (0.30)

SSB1 estimated from S_1 = 2008-2011 mean value

SSB2 estimated from S_2 = derived from the 2011 survey.

Table IX. Anchovy SSB in the Gulf of Cadiz by DEPM. Historical series.

Parameter	Total Gulf of Cádiz		
Eggs	2005	2008	2011
P_0 (eggs/m ² /day) (CV)	50.8(0.8) / 224.5(0.69)	184(0.44) / 348(0.35)	276 (0.32)
Z (day ⁻¹) (CV)	-0.039(0.75)	-1.43(0.29)	-0.29 (1.14)
P_{total} (eggs/day) ($\times 10^{12}$) (CV)	0.07(0.76) / 1.06(0.65)	0.31(0.44) / 1.80(0.35)	1.87 (0.36)
Surveyed area (km ²)	11982	13029	13107
Positive area (Km ²)	6139	6863	6770
Adults			
Female Weight (g) (CV%)	25.2(0.03) / 16.7(0.04)	23.67 (0.06)	15.17 (0.11)
Batch Fecundity(CV%)	13820(0.05) / 11160(0.05)	13.778 (0.07)	7486 (0.12)
Sex Ratio (CV%)	0.53(0.01) / 0.54(0.01)	0.528 (0.005)	0.53 (0.007)
Spawning Fraction (CV%)	0.26(0.07) / 0.21(0.07)	0.218 (0.065)	0.276 (0.04)
SSB			
Spawning Biomass –tons (CV)	14673	31527(0.32)	32757 (0.40)

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